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Flies as a Source of Enteric Pathogens in a Rural Village in Thailand

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The village of Ban Pong in northeastern Thailand was studied from January through December 1981 to determine the importance of flies as a source of enteric pathogens. The number of flies (predominantly *Musca domestica*) increased in kitchens and animal pens in the hot dry spring, when the incidence of diarrhea was highest in the village. Enterotoxigenic *Escherichia coli, Shigella* spp., non-O1 *Vibrio cholerae*, and *Vibrio fluvalis* were isolated from fly pools in yards (69%), animal pens (38%), bathrooms (35%), and kitchens (8%). Enterotoxigenic *E. coli* was isolated from one fly pool in May and from another in June, when the incidence of such infections was highest in the village. Flies often carry and presumably disseminate enteric pathogens in rural Thailand.

Diarrheal disease is endemic in developing tropical countries (22). Immunization (14), village hospitals (16), oral rehydration therapy (9), and sanitary intervention (2) have been proposed to solve this problem. However, the sources of bacterial enteric pathogens associated with patients with diarrhea (5) (enterotoxigenic *Escherichia coli* [ETEC], *Shigella* spp., vibrios, etc.) are still poorly understood. Before rational sanitary intervention can be introduced, the relative importance of enteric pathogens and their sources must be determined.

In 1981, a longitudinal study of enteric diseases in a small village in northeastern Thailand was performed (P. Echeverria, C. Tirapat, C. Charoenkul, S. Yanggratoke, and W. Chaicumpa, *in* Y. Takeda [ed.], Proceedings of the International Symposium on Bacterial Diarrheal Disease. Marcel Dekker, New York, 1983). This paper reports on one aspect of that study: the importance of domestic flies as a source of enteric pathogens.

MATERIALS AND METHODS

Study location. Ban Pong, a small farming village of 625 inhabitants in 125 homes, is located in Amphur Soongnern, Changwat Nakornrajsima, approximately 240 km northeast of Bangkok. This village is 15 km from a paved highway and is accessible only by dirt road. There are no rivers in Ban Pong, and inhabitants obtain water either from tube wells or a small pond at the Wat (temple), or by gathering rain water. The chief livelihood of the village is subsistence farming; most families keep animals (pigs, cows, buffalo, and ducks or chickens) in pens adjacent to or under their homes. The majority of homes have "suams" (concrete-encased bathrooms), which are flushed with water drawn from wells or the pond. From January through December 1981, inhabitants of Ban Pong were interviewed 6 days a week, and stools were collected from individuals with diarrhea to determine the prevalence of enteric pathogens. Temperature and humidity were measured each morning at the same location in the village.

Variation of fly density by month. Fly density was determined between 10 and 11 a.m. during the first week of every month. The number of flies alighting on a standard wooden grid (11) (90 by 90 cm in animal pens, yards, and suams and 44 by 44 cm in kitchens) in three 30-s time periods was recorded. Grids were set up 1 min before counting was started and picked up and repositioned between each count. Fly densities were determined in three animal pens, three yards, three suams, and three kitchens. The same areas were used each month. Results were expressed as the mean of nine 30-s counts performed at each of the four different locations. Counts on the smaller grid were multiplied by the ratio of the surface area of the larger grid divided by the surface area of the smaller grid (×4.18).

Identification and culturing of flies. Every 2 weeks approximately 75 flies were captured with a net in animal pens, yards, or suams, anesthetized by exposure to dry ice, and visually separated by species onto a clean paper surface. The number of each species was counted, and one of every five flies of a particular type was saved for later microscopic identification. The remaining flies were cultured as separate pools for each species. Because catching flies in kitchens with a net was impractical, fly paper (Tat fly paper; Walco-Linck Corp., Clifton, N.J.) was hung for 24 h. Flies were removed with sterile forceps and either cultured or saved in vials of xylene for later identification (a

preliminary study indicated that the glue that entrapped the flies was not bactericidal).

Flies were cultured by first washing them in 5 ml of brain heart infusion broth and immediately inoculating the broth onto MacConkey, Hektoen, and thiosulfate citrate-bile salts-sucrose media (Difco Laboratories, Detroit, Mich.). Brain heart infusion broth was inoculated into Hajna broth, alkaline peptone water (pH 8.0), and phosphate-buffered saline (pH 7.6). Alkaline peptone water was incubated for 6 h at 37°C and subcultured onto thiosulfate citrate-bile salts-sucrose medium. To identify *Yersinia enterocolitica*, brain heart infusion broth washes were inoculated into phosphate-buffered saline which was held at 4°C for 21 days and then subcultured on MacConkey and *Salmonella-Shigella* agar at 25°C for 48 h. Hajna broth was incubated at 37°C for 24 h and subcultured on Hektoen and desoxycholate media. Cultures were examined for *Salmonella, Shigella, Vibrio, Yersinia*, and *Aeromonas* spp. by standard procedures and with the use of the API 20E system (Analytab Products, Plainview, N.Y.) (4, 6, 15). Ten lactose-positive colonies selected from the MacConkey medium were stored on nutrient agar stab cultures and tested within 1 month of isolation for heat-labile (LT) and heat-stable (ST) toxin with Y1 adrenal (18) and suckling mouse (7) assays simultaneously. Non-O1 *Vibrio cholerae* were tested for cholera-like toxin production by GM1 enzyme-linked immunosorbent assay (17) and for ST toxin by suckling mouse assay (7).

Beginning in July 1981, the fly washings were inoculated onto nitrocellulose paper placed on a MacConkey plate and incubated at 37°C overnight. The filters were then successively treated with 0.5 N NaOH and 1.0 M ammonium acetate–0.02 N NaOH, air dried, baked at 65°C overnight, and examined for nucleotide sequences coding for LT and ST by DNA hybridization assay as previously described (13).

RESULTS

Fly population. Approximately 90% of the flies were identified as *Musca domestica* Linnaeus, 6% as *Musca sorbens* Wiedemann, and 2% as *Phaenicia cuprina* Wiedemann, and 2% were distributed among six other types of flies (*Lymonophora* spp., *Chrysomyia megacephala* Fabricius, *Sarcophagidae* spp., *Sepsidae* sp., *Tabanidae* sp., and *Stomoxys calcitrans* Linnaeus). The relative proportion of each species of fly found at different locations each month did not differ appreciably throughout the year. *M. domestica* was consistently dominant, making up 96% (262 of 274) of flies caught from kitchens, 92% (341 of 371) from animal pens, 87% (306 of 350) from suams, and 87% (311 of 359) from yards.

The mean number of flies counted at 12 different locations (three kitchens, three suams, three yards, and three animal pens) each month is shown in Fig. 1. The number of flies in kitchens increased in March, remained at this level in April, and declined in May. The number of flies in animal pens increased in April, was highest in June, and decreased in July. These increases in numbers of flies were not associated with an increase in the prevalence of any single species. The temperature and humidity recorded each month in Ban Pong are shown in Table 1.

Bacteriological results. As shown in Table 2, ETEC, *Shigella flexneri*, non-O1 *V. cholerae*, and *Vibrio fluvalis* were isolated from 69% of the fly pools in yards, 38% of fly pools in animal

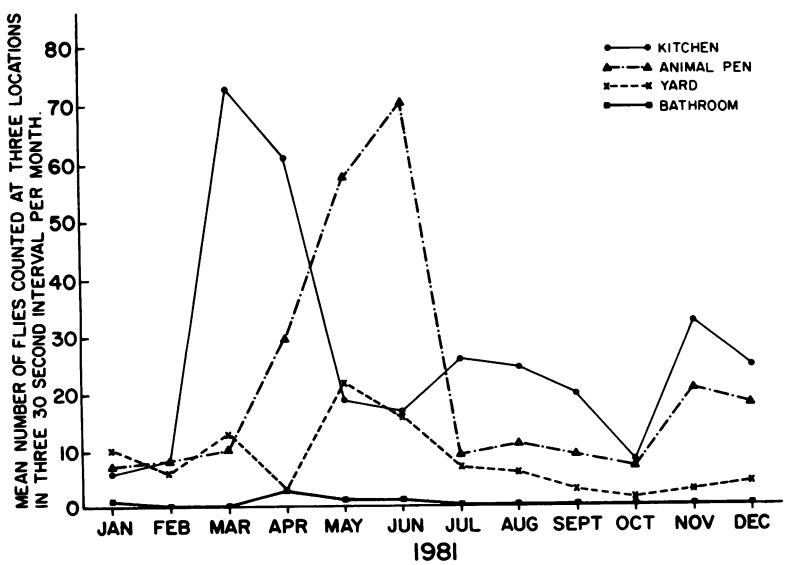

FIG. 1. Mean number of flies counted in three animal pens, three yards, three bathrooms, and three kitchens each month in Ban Pong in 1981.

TABLE 1. Mean monthly temperature and humidity in Ban Pong in 1981

Month	Temp (°F)	Humidity (%)
January	64	68
February	79	58
March	94	48
April	92	55
May	84	70
June	81	74
July	81	79
August	81	81
September	79	83
October	75	83
November	67	67
December	66	66

pens, 35% of fly pools in suams, and 8% of fly pools in kitchens. *Salmonella* spp. and *Y. enterocolitica* were not isolated from any fly pools. All enteric pathogens were isolated from the housefly, *M. domestica*. Of 15 non-O1 *V. cholerae*, 2 produced cholera-like toxin, as determined by GM1 enzyme-linked immunosorbent assay. Although *E. coli* was isolated from 88% of pools of flies in yards, 85% of fly pools in animal pens, 81% of fly pools in suams, and 68% of fly pools in kitchens, ETEC was isolated only from pools of flies caught in one yard in June and one kitchen in May. ETEC from these two pools produced LT alone and were of different serotypes, O?:K?:H- and O6:K12:H?. DNA encoding for LT or ST was not found in 32 fly pools collected after July 1, 1981. *Aeromonas hydrophila*, a bacteria of uncertain enteropathogenicity, was isolated from 63% of the fly pools.

The number of inhabitants with diarrhea in Ban Pong and the percentage of individuals with diarrhea infected with different enteric pathogens per month is shown in Fig. 2. In 1981, ETEC was isolated from 11%, *S. flexneri* from 8%, nonO1 *V. cholerae* from 1.5%, and *A. hydrophila* from 34% of 132 inhabitants with diarrhea in Ban Pong. *V. fluvalis* was not isolated from any inhabitants with diarrhea (P. Echeverria, C. Tirapet, C. Charoenkul, S. Yanggratoke, and W. Chaicumpa, *in* Y. Takeda [ed.], Proceedings of the International Symposium on Bacterial Diarrheal Disease. Marcel Dekker, New York, 1983).

DISCUSSION

The common housefly, *M. domestica*, was recently documented as the most abundant species of fly in marketplaces, garbage, slaughter houses, and animal sheds in the northern, northeastern, and central parts of Thailand (21). This species of fly was by far the most abundant throughout the year in the kitchens, yards, animal pens, and suams of Ban Pong village during our study. Furthermore, the densities of this and other species of fly increased significantly in kitchens and animal pens during the hottest, driest season of the year, when diarrheal disease was most prevalent among the villagers.

Bacteria that have previously been associated with episodes of diarrhea were isolated from *M. domestica* in all locations in Ban Pong. Flies caught in yards were more often colonized with enteric pathogens (69%) than were those from animal pens (38%), suams (35%), or kitchens (8%). ETEC was isolated from only one fly pool in May and from another in June, when the incidence of ETEC infections was highest in inhabitants with diarrhea. Although only a small proportion (2 of 144) of the pools examined contained flies which carried ETEC, this study demonstrates that flies carry and presumably disseminate this pathogen. Further studies are necessary to determine the importance of flies as carriers of ETEC.

V. fluvalis was the most common bacterial enteric pathogen isolated from *M. domestica*. These organisms have been implicated as a cause of diarrhea worldwide (1, 8, 10, 12, 19) and

TABLE 2. Pools of flies containing enteric pathogens

Enteric pathogen	No. of pools				
	Yard (26)[a]	Animal pen (26)	Bathroom (26)	Kitchen (66)[c]	All sites (144)
ETEC	1	0	0	1	2
Shigella spp.	0	1	0	0	1
Non-O1 *V. cholerae*	7	4 (1)[b]	3 (1)[b]	1	15
V. fluvalis	10	5	6	3	24
Total	18 (69)[c]	10 (38)[c]	9 (35)[c]	5 (8)[c]	42 (29)[c]
A. hydrophila (possible enteric pathogen)	20	17	22	32	91 (63)[c]

[a] Total number of pools. More pools of flies were collected in kitchens than at other locations since it was often necessary to hang fly paper to collect 75 flies every 2 weeks.

[b] Number of non-O1 *V. cholerae* which produced *V. cholera*-like toxin as determined by GM1 enzyme-linked immunosorbent assay.

[c] Percentage of pools containing an enteric pathogen.

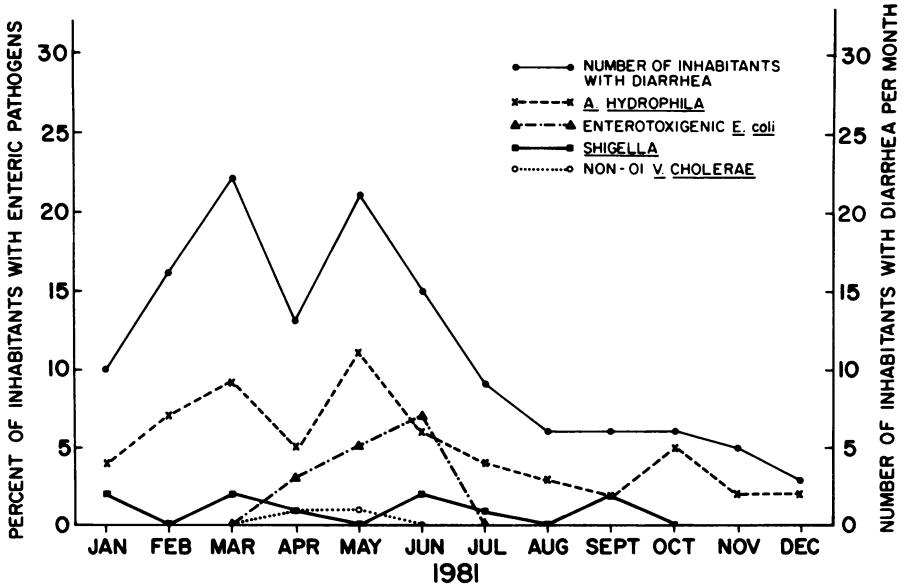

FIG. 2. Inhabitants with diarrhea associated with different enteric pathogens in 1981.

have been isolated from a variety of environmental sources (19). Four patterns of biological activity have been found in non-O1 *V. cholerae* isolated from patients with diarrhea in Bangladesh (20): those that produce a cholera-like toxin, those that produce an ST toxin as measured in the suckling mouse assay, those whose whole cultures produce enteritis in infant rabbits, and others that demonstrate no activity at all. Spira and Daniels (19) found that 6 of 18 strains were isolated from patients with diarrhea, but none of an equal number of environmental isolates produce a cholera-like toxin. The enteropathogenicity of non-O1 *V. cholerae* is not fully understood. However, 13% (2 of 15) of isolates from flies produced a cholera-like toxin and were presumably capable of causing diarrhea in humans.

V. fluvalis, which has been isolated from patients with severe cholera-like diarrhea in Bangladesh (5), and *S. flexneri*, a well-accepted enteric pathogen, were cultured from flies in Ban Pong. Flies have previously been implicated in the dissemination of *Shigella* spp. in crowded, unsanitary conditions (23), but environmental sources of *V. fluvalis* are unknown. *A. hydrophilla*, which has been suggested but not proven to be an enteric pathogen (15), was commonly isolated from flies. The significance of the high prevalence of *A. hydrophila* in fly pools in unknown. However, the data in Fig. 2 suggest a direct correlation between *A. hydrophila* isolates from inhabitants and the number of inhabitants with diarrhea.

Although it was impossible to determine the relative importance of flies versus other routes in the dissemination of enteric pathogens, several conclusions can be drawn from this study: (i) the majority of flies in yards, animal pens, suams, and kitchens in Ban Pong were *M. domestica*; (ii) both the fly population and the incidence of diarrhea increased in the hot dry season; (iii) 29% of fly pools collected in the village carried enteric pathogens; (iv) flies caught in yards, suams, or animal pens carried enteric pathogens more often than those caught in kitchens; and (v) ETEC was isolated from flies wen ETEC infections were most frequent among village inhabitants with diarrhea. Efforts to decrease the fly population or reduce their numbers in areas in which people live and eat would be a reasonable approach to decreasing diarrheal disease in rural Thailand. Prospective studies performed before and after active interventions to reduce the fly populations may be required to determine the importance of flies in disseminating enteric pathogens. Our work suggests that such a study would be worthwhile.

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